A MORPHOLOGICAL STUDY OF DIOSPYROS VIRGINIANA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 145

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(WITH PLATES I–III)

Diospyros virginiana, the northernmost representative of the tropical family Ebenaceae, grows abundantly in the southern states and as far north as the southern part of Illinois and Indiana. Cultivated trees are found also in the extreme northern part of those states. No report of any morphological work upon this family has been found, except a brief paper on "The seedless persimmon" in the report of the Proceedings of the Indiana Academy of Science for 1908. The material for this investigation was collected in 1906 and 1908 from cultivated trees at Decatur (Illinois), and from native trees near Springfield (Missouri), Topeka (Kansas), and Memphis (Tennessee).

Floral development

The winter buds are composed of numerous tough hairy scales enveloping a very rudimentary shoot. The flower buds develop upon this shoot during its rapid growth in the spring. At Decatur, in 1906, the buds began to swell and to become green the latter part of April. Young shoots gathered the first week in May bore flower buds in the early stages of development. On May 30 the shoots were 20 cm. or more long and the flowers were beginning to open.

So far as the trees from which material was collected were observed, they were dioecious and bore only imperfect flowers. One possible exception has been found recently. Near Auburn (Indiana) there is a cluster of staminate trees, originating apparently from one tree, that are reported to have borne fruit occasionally. The flowers were carefully examined in the spring of 1910, and no variations from the regular staminate type were found. Unless a pistillate tree has been cut away, it seems prob-

able that perfect flowers are borne some seasons, as has been reported from Kansas.[1]

The staminate flowers are smaller than the pistillate and in clusters (fig. 1), 16 fertile stamens surrounding the sterile pistil. The pistillate flowers are solitary, and usually contain 8 sterile stamens, but very often the number is greater.

The early stages of the development of the two flowers are the same. The floral cycles are generally preceded by a pair of bracts, though often there is only one (fig. 2). The calyx next appears (fig. 3) and becomes a massive enveloping cup before the other cycles can be seen, which appear in centripetal sequence (fig. 4). The corolla can be distinguished before the stamens, but they develop together in the typical sympetalous fashion. Occasionally the calyx or corolla has more than four parts; this is illustrated in fig. 6, in which the calyx has five divisions.

The stamens of the staminate flower fork (fig. 5), thus doubling the pistillate number (fig. 6); in the pistillate flower it is a common occurrence to find the number increased by the branching of one or more of the stamens. The fertile pistil contains eight ovules. The style is single, but the stigma is four-parted. Not many sterile pistils were examined; those that were had no ovules and a short imperfect style.

Megasporangium and megaspores

The ovule is anatropous and has two integuments (fig. 7), this last character being unusual among the Sympetalae. The mother cell can be distinguished by its size and conspicuous nucleus about the time the inner integument is first visible (fig. 8). Judging by the repeated appearance of this stage in the material, it is especially persistent. Only one mother cell occurs in a sporangium, and is always next to the outer layer of nucellar cells, no parietal cell being cut off. One complete figure of the first division of the mother cell was found in the spindle stage (fig. 9). Compared with the preceding conspicuous nucleus of the mother cell, the spindle is small and has very small and numerous chromosomes.

[1] The Industrialist, Kansas State Agricultural College, March 1904.

A portion of another figure showing the formation of the wall between the daughter cells was seen.

Four megaspores are formed in a linear row (figs. 10, 11), but it is not always complete, three cells not being uncommon. One exception to the usual arrangement was discovered, in which the outer daughter cell is divided by a vertical wall (fig. 12). As usual, the chalazal megaspore becomes the embryo sac (fig. 13).

Early stages of the embryo sac

The two and four-celled stages of the embryo sac were not found. At the eight-celled stage the sac is small, much longer than wide, and somewhat pointed at the micropylar end. When the sac is seen enveloped by the single nucellar layer, it appears decidedly cone-shaped, and is supported upon a stalklike portion of the nucellus as upon a pedestal (fig. 15). The growth of the sac does not obliterate this nucellar tissue until a comparatively late stage of the ovule. In the sac of fig. 14, which is the eight-celled stage in which the polars are differentiated, only seven nuclei are shown, and it seems quite probable that that is the full number for that particular sac, because there is much evidence that the usual number of nuclei is not always present. This conclusion is reached because of the conspicuous absence of antipodals. Three antipodals were found in one sac, but only after a long search. Extremely early disintegration would also account for the absence of these cells, but no evidence was found for this explanation.

The egg apparatus in the eight-celled stage shows nothing unusual. The three cells are in the ordinary position, and there is the customary differentiation of the cells in size.

In striking contrast to these two groups, the antipodals and egg apparatus, are the polars, which are large and conspicuous (figs. 21*a*, 21*b*), and are found either approaching or fusing in material gathered during the flowering time.

During the development of the sac the integuments become massive, and the innermost layer of cells of the inner integument becomes large and full of protoplasm, forming a tapetal layer com-

pletely enveloping the sac and extending around the stalklike nucellus and far up the micropyle (fig. 16).

The study of the sac is made difficult by the dense outer integument, through which killing fluids penetrate with difficulty, and also by the presence of chemicals which interfere with the stains. This last difficulty is especially true of the micropylar end of the sac, which when mature becomes a beaklike accumulation of a mucilaginous substance.

Pollination and fertilization

These studies have so far revealed only doubtful evidence of pollination and none at all of fertilization. Careful search has failed to show pollen tubes in the tissue of the style. The most positive evidence has been a few cases in which the mucilaginous substance has been divided in such a manner as to suggest a pollen tube penetrating the sac, and a few others in which there is the appearance of a swollen tip of a pollen tube within the sac. This evidence is discredited because the mucilaginous substance has been seen similarly divided too early for pollination, and the resemblance to the swollen tip of a pollen tube may be due to an incomplete or imperfect section of the stage shown in figs. 17*a* and 17*b*. Other slight evidence may be found in the presence of the spindle and chromosome-like bodies of figs. 19 and 20*b*. These may possibly have entered the sac by way of the pollen tubes or may have originated from the nuclei of the tube. The fusing polars which are so conspicuous have been carefully examined for a third nucleus but none has been seen.

The doubtful character of this evidence has naturally raised the question, whether pollination is essential to fruit and seed production. The field observations relating to pollination are limited and not very exact, but they suggest the possibility of an interesting problem. A tree in Decatur, from which material was collected in 1906, bears seeded fruit abundantly, though no staminate tree is known to be nearer than two miles. In order to see if the pollen was carried that distance or was essential, a branch was covered in the spring of 1909 during flowering time so as to prevent the

access of the bees. No fruit was borne on this branch, but was developed upon the neighboring ones. The details of this experiment cannot be vouched for, but until more careful ones are tried it is affirmative evidence for pollination.

On a fruit farm beyond the city limits of Decatur is a cluster of 6 or 8 trees, the largest of which is the parent of the smaller ones. The gardener reports that seedless fruit occurs on all these trees, but not in the same proportion. The fruit of the largest tree is usually many-seeded and only rarely seedless, but some of the smaller trees bear few-seeded and seedless fruit abundantly. No differences were noted among the flowers of these trees, or any when the prepared material was compared with that from native trees. The later stages are yet to be examined, for no collections have been made from the cluster after the flowering time.

The nearest staminate tree is not known. One was reported within a quarter of a mile, but two careful examinations of the region have failed to locate it. Since persimmon trees in bloom always swarm with bees, they are doubtless the pollinating agent. It does not seem probable that the bees avoid certain trees, but it is possible that the supply of pollen which they carry is limited, and is deposited most freely on those trees which from their position they visit first. The trees of the cluster which bear the larger proportion of abnormal fruit are the least exposed trees.

That the distance of the staminate trees does make a difference in the fruit is reported in the 1907 Yearbook of the Department of Agriculture, in which one variety is mentioned as characteristically few-seeded, and the observation made that this and other varieties have fewer seeds when grown at a distance from staminate trees.

In 1910 the Decatur trees were again visited. A severe frost in May killed the first buds, consequently the conditions that season were not normal. The city trees bore no fruit at all, and the cluster only a small proportion of its normal amount. The fruit on all the trees was smaller than usual, inclined to early decay, and almost wholly seedless. One lot of 33 contained only one seeded fruit; another of 12, two. The embryos were normal. This state of affairs suggests an indifference to fertilization, even

to pollination, as the stimulating cause of the development of the ovary into the characteristic persimmon fruit.

In the paper previously referred to on "The seedless persimmon," the seedless fruit is reported to occur most abundantly on the lowest branches of the trees. No differences were found in the flowers; all had a fertile pistil and sterile stamens. The examination of the embryo sacs brought out no evidence of pollination or fertilization. In one case cited, if pollen is transferred from the staminate trees, the bees must carry it three or four miles; this was not determined. Perfect flowers are suggested as a possible source of the pollen. Any attempt to explain or to suggest the problems of pollination involved is impossible until further observations are made.

Late stages of embryo sac; endosperm and embryo

The absence of evidence of pollination and fertilization has made impossible at present a connected account of the series of events in a normal seed-producing sac following the eight-celled stage, nor can these stages be surely identified, because the ovules of the seeded fruit frequently fail to develop into seeds, and since the normal course has not been determined, it is uncertain where the two diverge and what the differences are.

The entire ovule increases very rapidly in size after the corolla falls off, but no sign of an embryo was found for a number of weeks afterward. Material sent from Memphis is past blooming the latter part of May, but not until the last of June or the first of July can embryos be found easily in the fresh material. Long before this the sac has become densely filled with endosperm. Judging from the size of the sac, the first division of the primary endosperm nucleus follows closely on the fusion of the polars, and the other divisions follow rapidly after this (figs. 22, 23*a*, 23*b*).

The antipodal nuclei disappear after the eight-celled stage, but the micropylar nuclei undergo interesting changes. In fig. 17*a* there are three protoplasmic masses very distinctly differentiated. The middle one contains numerous, rather large, spherical, densely staining bodies. Because of its size, position, and

persistence in older sacs, this mass is surely the egg and the two other masses the synergids. Fig. 17*b* is another view of the same sac and shows the synergids more distinctly. Fig. 16 undoubtedly shows the two synergids, but no division of the surrounding protoplasm. In fig. 18 the egg appears with the spherical bodies regularly arranged; the small nucleus is a synergid. Fig. 19 is the micropylar end of a sac in which figures of dividing endosperm nuclei were seen. The large cell is the egg, near which are the spindle-shaped figures mentioned before. From their position it is possible that they are the remains of the disintegrating synergids. The large cell of fig. 24 is the egg at a later stage than the other figures show, because it is almost completely enveloped by the endosperm; numerous illustrations of this stage were found. No more distinct segmentation of the egg was seen than appears in fig. 25, in which the protoplasm is divided, but only one nucleus could be found, which makes it a doubtful case. Even after the egg is completely surrounded by endosperm, the deeply staining globules remain, but they and the whole egg seem to lose the prominence shown in fig. 24. However, this is partly relative owing to the increased size of the whole ovule. Figs. 20*a* and 20*b* show the curious chromosome-like bodies in the micropylar end of the sac; they are rodlike and twisted, resembling chromosomes, but not those of *Diospyros*, which are very small. The investigation of these phases of the life history of the persimmon will be continued in the hope that the complete sequence of events in the embryo formation will be found.

The youngest unmistakable embryo that has been seen consists of three cells (fig. 26). This embryo was in the extreme micropylar end of the sac, imbedded in endosperm. Its position agreed very nearly with that of the egg when surrounded by endosperm, but no proof could be found that it originated from that cell. Fig. 27 represents the embryo at a much later stage, but does not yet show differentiation into stem tip and cotyledons. Fig. 28 is a variation from the common type, and fig. 29 is the appearance of the embryo about the time it can be distinguished without a lens. One case of polyembryony was found (fig. 30), and one lot of material contained freak embryos, one of which is shown in

fig. 31, in which a second pair of cotyledons has developed upon one of the original pair.

Microsporangium and pollen

The studies of the stamens and pollen were made from material collected in 1906 from a single tree near Decatur. This tree bloomed a few days later than the pistillate trees from which collections were being made at the same time. The pollen formation was easily traced. The only difficulty encountered was in the late stages when the protoplasm is dense and evidently contains the same chemicals that interfere with the stains in the embryo sac.

Each stamen produces four sporangia, whose early stages were not traced because the earliest collections were made May 28, about a week before the flowers opened. At that date the sporangia contained large pollen mother cells surrounded by a single tapetal layer (fig. 33). The division into tetrads is shown in figs. 34 and 35. The figures are small and the chromosomes numerous, 30 at least. In the mature pollen grain more than one nucleus could be rarely distinguished, and that one not nearly so conspicuous as the nucleus of the tetrad (figs. 36, 37). It is very possible that the dense protoplasm frequently obscures the second small nucleus. A considerable difference in the size of the pollen grains was noted; this and the frequent presence of a single nucleus, together with the lack of proof of pollination, raise the question of the fertility of the pollen. This remains to be determined along with the other problems of pollination.

Summary

1. The flowers are developed on shoots of the same season's growth. The floral cycles appear in the following order: a pair of bracts, the calyx, the corolla and stamens, and lastly the pistil.

2. The ovule is anatropous and has two integuments. A single mother cell is formed beneath the outermost layer of the nucellus, from which four megaspores develop, the chalazal one becoming the embryo sac.

3. The embryo sac at the eight-celled stage is small, somewhat pointed at the micropylar end, and rests upon a stalklike portion of the nucellus. A tapetal layer of cells from the inner integument completely surrounds it. The egg apparatus in this stage is not conspicuous; the polars are large and striking in appearance; the antipodals are found with so much difficulty that it is probable that one or more of the cells is often lacking.

4. The studies of pollination and fertilization are not complete. Little evidence of pollination has been found and none of fertilization. The production of seedless fruit is probably involved in the problem of pollination.

5. After the flowers fall, the whole ovule increases rapidly in size. The egg enlarges and becomes filled with densely staining globules. The primary endosperm nucleus divides early and the endosperm fills the sac, and then crowds the inner integument quite up to the dense outer one.

6. The embryo is late in appearing. The earliest stage identified was a three-celled one in the extreme micropylar region. The tendency to variation seen in many of the stages is shown here in the two types found, the freak embryos and the case of polyembryony.

7. Pollen mother cells were found on a tree a week before the older flowers opened. The mother cells are large and the whole mass is surrounded by a single tapetal layer. The spindle in the tetrad formation is small, the chromosomes being 30 or more. The pollen grains show some difference in size, and frequently only one nucleus could be distinguished.

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EXPLANATION OF PLATES I–III

FIG. 1.—Diagram of a cluster of staminate buds.

FIG. 2.—A pistillate bud, showing enveloping bracts (*b*).

FIG. 3.—A pistillate bud, showing a single bract (*b*) and the beginning of the calyx.

FIG. 4.—A pistillate bud, showing calyx (*k*), corolla (*c*), stamens (*s*), and pistil (*p*).

FIG. 5.—A staminate flower, showing calyx (*k*), corolla (*c*), the two stamens (*s*), and pistil (*p*).

FIG. 6.—A cross-section of a pistillate flower, showing the unusual division of the calyx into five parts, the union of the corolla, and the four parts of the pistil; diagram.

FIG. 7.—An ovule with the two integuments.

FIG. 8.—The nucellus containing the mother cell, and showing the beginning of the inner integument (*i*).

FIG. 9.—First division of the megaspore mother cell.

FIG. 10.—The two daughter cells.

FIG. 11.—The four megaspores.

FIG. 12.—The four megaspores; the outer daughter cell divided by a vertical wall.

FIG. 13.—The functioning megaspore; the others disintegrating.

FIG. 14.—Embryo sac; polars differentiated.

FIG. 15.—Diagram of an ovule showing the relative size of the parts, the shape of the sac (*e*), the stalklike nucellus (*n*), and the massive integuments (*i*).

FIG. 16.—The micropylar end of the embryo sac, showing the synergids and the enveloping tapetal layer.

FIG. 17*a*.—Micropylar end of sac; synergids and egg.

FIG. 17*b*.—Same sac as 17*a*, showing fusing polars.

FIG. 18.—Micropylar end of sac; one synergid and the egg.

FIG. 19.—Micropylar end of sac; egg and spindle-shaped bodies.

FIG. 20*a*.—A detail of the micropylar end of a sac, showing the chromosome-like bodies and the egg filled with the densely staining globules.

FIG. 20*b*.—Same as 20*a*; chromosome-like bodies more clearly shown.

FIG. 21*a*.—Fusing polars.

FIG. 21*b*.—Fusing polars.

FIG. 22.—First division of the primary endosperm nucleus.

FIG. 23*a*.—Division of endosperm nucleus.

FIG. 23*b*.—Division of endosperm nucleus.

FIG. 24.—Egg almost surrounded by endosperm.

FIG. 25.—Egg; segmentation suggested.

FIG. 26.—Young embryo.

FIG. 27.—Young embryo.

FIG. 28.—Variation of type of embryo.

FIG. 29.—Shape of embryo at the time it can be seen without a lens; diagram.

FIG. 30—Polyembryony; diagram.

FIG. 31.—A freak embryo; a second embryo (c^2) developing on one of the first pair of cotyledons (c^1); diagram.

FIG. 32.—Diagram of a cross-section of an anther, showing the four sporangia.

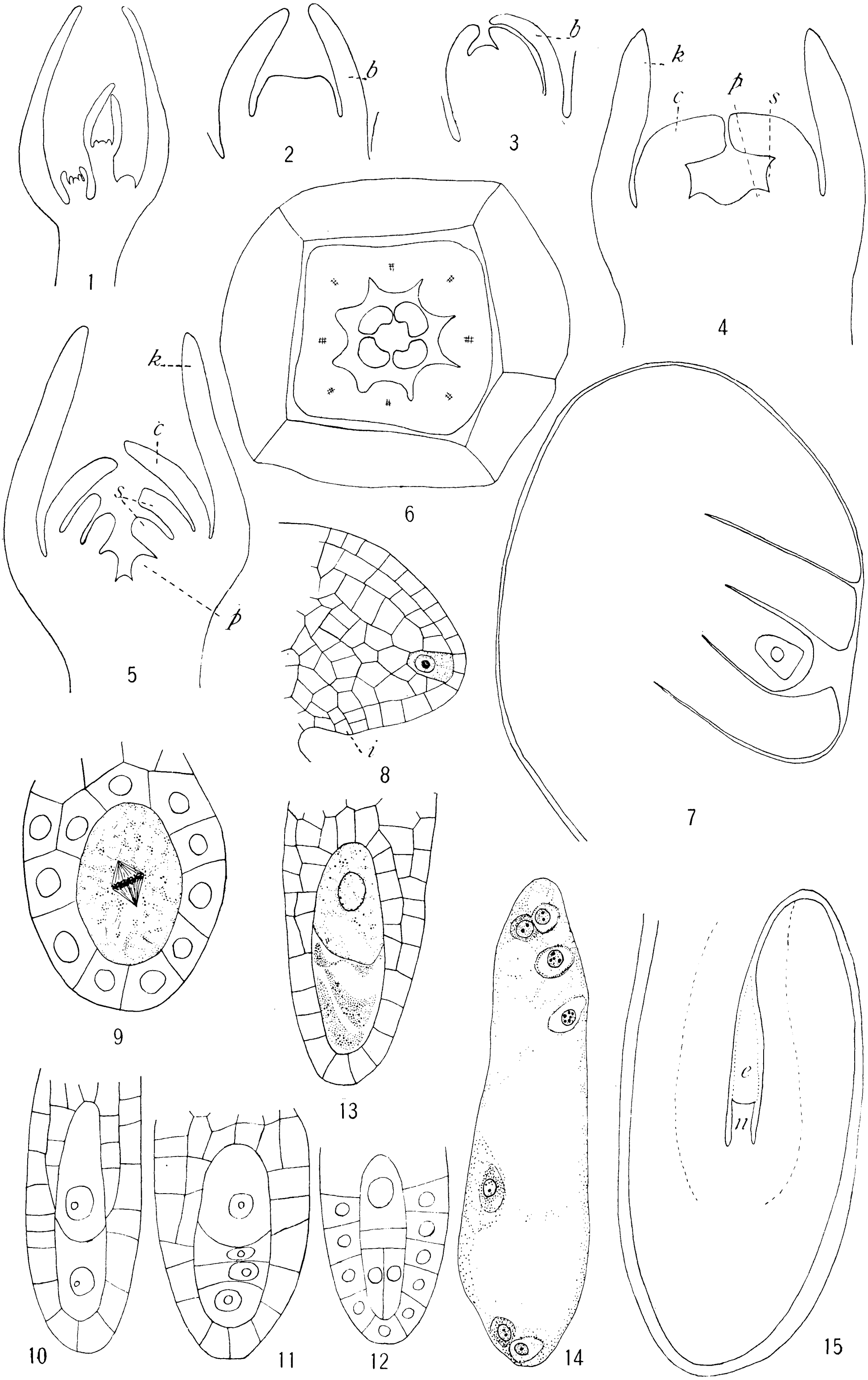
FIG. 33.—Pollen mother cells.

FIG. 34.—Formation of tetrads.

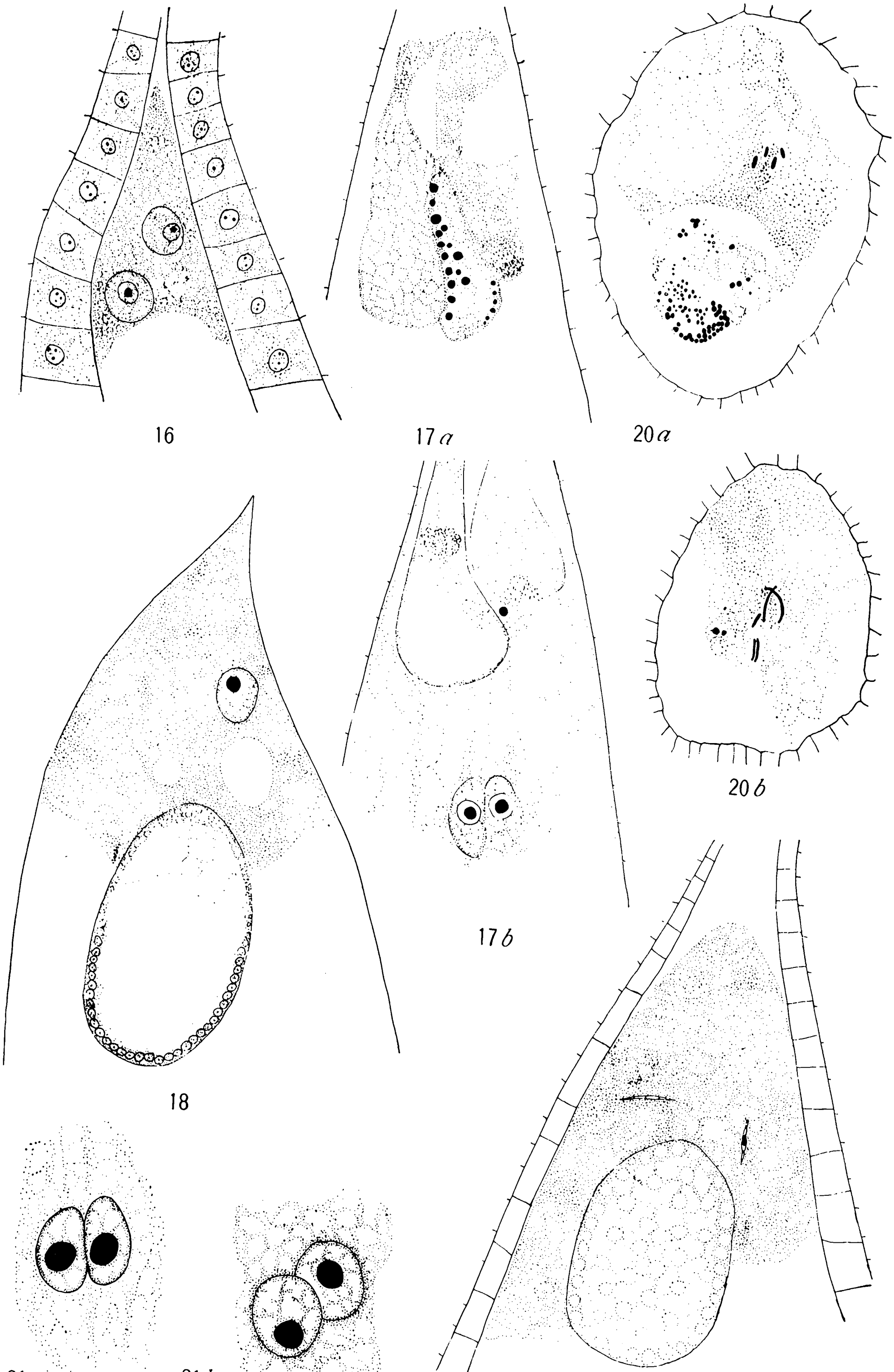
FIG. 35.—Tetrads.

FIG. 36.—Pollen grain; one nucleus.

FIG. 37.—Pollen grain; two nuclei.



HAGUE on DIOSPYROS



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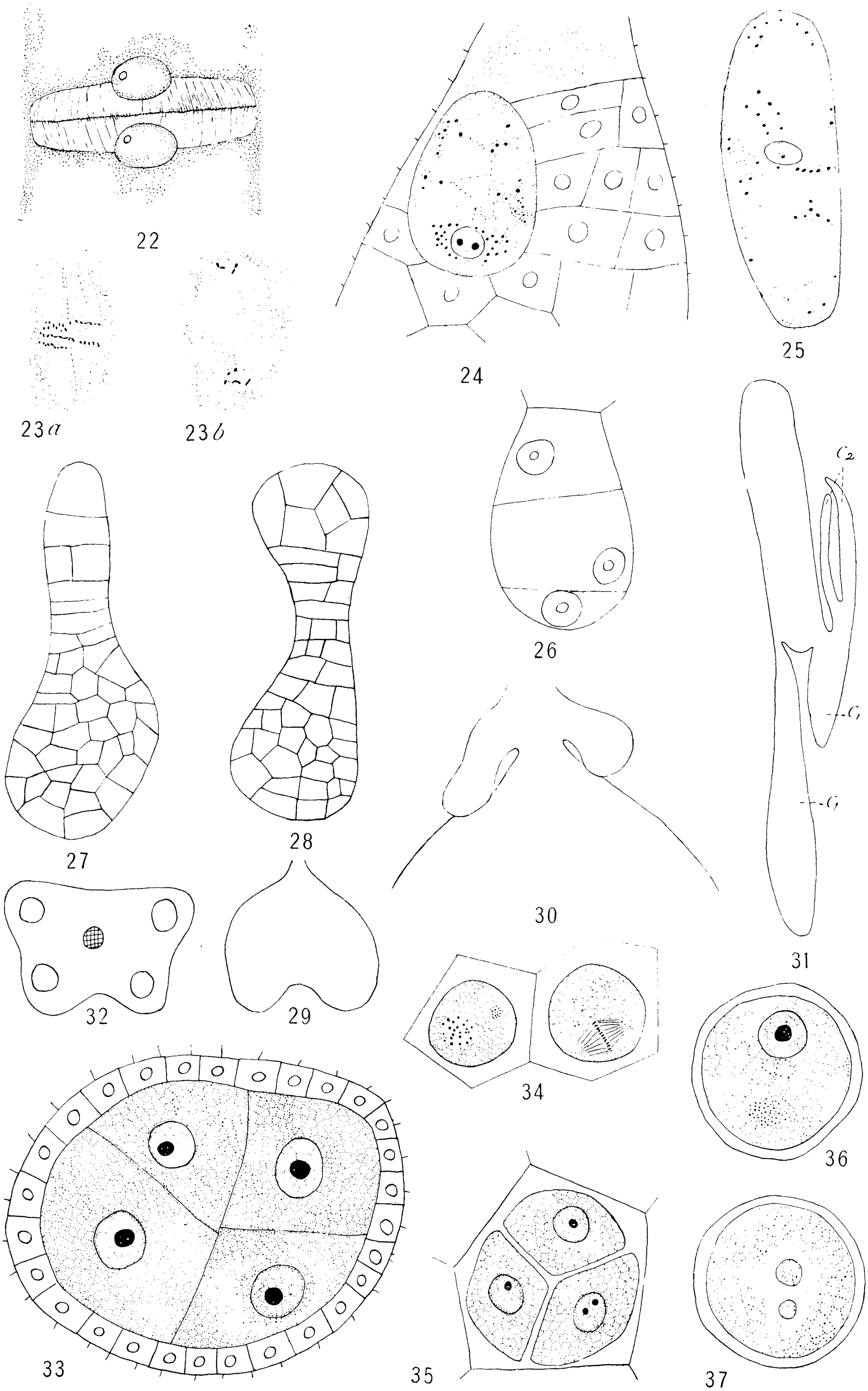

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